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Research Article
Molecular Characterization of Tomato Yellow Leaf Curl Virus (TYLCV) Infecting Pepper and Common Bean

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Abstract

Background: Tomato Yellow Leaf Curl Virus (TYLCV) is a circular single strand DNA begomovirus, which causes one of the most important diseases in tomato worldwide. This virus was observed on tomato plants for first time in Saudi Arabia in 1978 and has been endemic since then. **Materials and Methods:** Plant samples of pepper (*Capsicum frutescens*) and common bean (showing leaf yellowing and curling) were collected from Jizan and Hail Southwest and North of Saudi Arabia, respectively. Rolling Circle Amplification (RCA) was used to amplify the complete genome for two TYLCV strains (TYLCV-GB and TYLCV-Pep). The amplified complete genomes of TYLCV-GB (2779 bp) and TYLCV-Pep (2764 bp) were cloned, sequenced and registered in the GenBank database under accession numbers KF444467 and KF435136, respectively. **Results:** Phylogenetic analyses revealed that the length of the Intergenic Region (IR) was 305 bp for TYLCV-GB and 290 bp for TYLCV-Pep. The homology tree showed that TYLCV-GB and TYLCV-Pep belong to two different clusters and the identity between them was 81.2%. The TYLCV-GB clustered with isolates from Sudan, whereas TYLCV-Pep clustered with isolates from Iran. Six Open Reading Frames (ORFs) (V1, V2, C1, C2, C3 and C4) were found on the TYLCV-Pep dsDNA replicative form while seven ORFs (V1, V2, C1, C2, C3, C4 and C5) were found on the TYLCV-GB genome. The DNA β satellite was found to be associated with TYLCV-GB but not with the TYLCV-Pep. **Conclusion:** This study provide definitive evidence based on molecular data suggesting that TYLCV-GB and TYLCV-Pep are two different strains in Saudi Arabia with different invasion routes.

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato Yellow Leaf Curl Virus (TYLCV) causes one of the most devastating viral diseases in tomato plants (*Solanum lycopersicum*) worldwide (Czosnek and Laterrot 1997; Hanssen *et al.*, 2010). The TYLCV (family Geminiviridea, genus *Begomovirus*) was first observed in tomato plants more than 70 years ago in the eastern Mediterranean (Antignus and Cohen, 1994; Lapidot and Friedman, 2002; Polston *et al.*, 2006). Recently, seven genera have been recognized within the family Geminiviridea according to insect vector, genome arrangement and sequences identity (Brown *et al.*, 2012; Adams *et al.*, 2013; Martinez-Ayala *et al.*, 2014). The genus *Begomovirus* is considered the most prevalent worldwide within this family. These viruses infect dicotyledonous plants and are transmitted in nature by the whitefly *Bemisia tabaci* (De Barro *et al.*, 2011). The majority of the members of this genus are clustered in two main phylogenetic clades called old and new world (Briddon *et al.*, 2010). Most of the old world members have monopartite genome with one genomic component of ~2.8 kb (DNA-A). while the new world members have bipartite genomes with two genomic components of ~2.6 kb each (designated DNA-A and DNA-B), except Tomato leaf deformation virus (ToLDeV) recently reported from Peru as monopartite begomovirus (Sanchez-Campos *et al.*, 2013; Melgarejo *et al.*, 2013; Martinez-Ayala *et al.*, 2014). Sub-viral molecules have been found associated with some monopartite viruses from the Eastern Hemisphere called betasatellites (DNA-β), which encode a single protein on the complementary-sense strand, referred to as βC1 (Briddon *et al.*, 2002, 2003; Idris *et al.*, 2012). The βC1 protein has been shown to function as a virulence factor of disease severity and symptom development (Briddon *et al.*, 2001; Cui *et al.*, 2004).

Recently, TYLCV has been detected in tomato plants from different locations in Saudi Arabia (Alhudiab *et al.*, 2014). Recent studies based on molecular analyses has resulted in the characterization and elucidation of the genomic sequence (Alhudiab *et al.*, 2014). The TYLCV was observed to infect not only tomato but also other cultivated crops including (*Capsicum* spp.) (Morilla *et al.*, 2005; Polston *et al.*, 2006) and common bean (*Phaseolus vulgaris*) (Navas-Castillo *et al.*, 1999; Lapidot and Friedman, 2002; Papayiannis *et al.*, 2007; Segundo *et al.*, 2008; Kamaal *et al.*, 2015). In addition, TYLCV infects various weeds such as henbit (*Lamium amplexicaule*) (Kil *et al.*, 2014). There is little information and evidence available on TYLCV infection of pepper and common bean crops. Most of this evidence reported for TYLCV infection of *C. annuum* and *P. vulgaris* while only one study reported *C. frutescens* to be a host for TYLCV (Polston *et al.*, 2006). In this study, this study report the molecular characterization of 2 different strains of TYLCV that naturally infect *C. frutescens* and *P. vulgaris* in Saudi Arabia. This study provides definitive evidence based on molecular data and nucleotide sequence that beta satellite (DNA-β) was associated with TYLCV isolated from *P. vulgaris*.

MATERIALS AND METHODS

Sample collection: Plant samples of pepper (*Capsicum frutescens*) and common bean (*Phaseolus vulgaris*) showing yellowing and leaf curling symptoms were collecting from two different locations of Saudi Arabia, Jizan (Southwest of Saudi Arabia) and Hail (North of Saudi Arabia) during 2012-2013. Samples were kept in cool boxes after collecting from the field and stored at -80°C or used directly for DNA extraction.

Total DNA extraction and PCR detection: Total DNA was extracted from the leaves samples using DNaesy plant mini extraction kit (Qiagen) and according to its manufacturer's guidelines. Polymerase Chain Reaction (PCR) was used to detect *Begomovirus* infection in the collected samples using the degenerate primer pair AV_{core}/AC_{core} (Brown *et al.*, 2001). These primers amplify fragments of DNA-A of ~580 bp (Brown *et al.*, 2001). The PCR reactions were done in a Techne TC-4000 Thermal Cycler. Each PCR reaction contained 2.5 μL of extracted DNA, 0.25 mM $MgCl_2$, 0.25 mM dNTPs, 2.5 μM of each primer (AVcore/ACcore), 1×enzyme buffer and 0.5 U of Taq DNA polymerase in 25 μL final volume. The PCR amplification program started with an initial denaturing step at 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, 50°C for 2 min and 72°C for 2 min and a final extension step at 72°C for 10 min. The amplified products were determined on 1.5% agarose gels in TBE buffer (tris-boric acid- EDTA) and stained with ethidium bromide (0.5 mg mL^{-1}).

Rolling Circle Amplification (RCA): Total nucleic acids were extracted from symptomatic leaves of pepper and common bean using DNaesy plant mini extraction kit (Qiagen) as mentioned above. Extracted DNA was subjected to TempliPhi amplification (Amersham Biosciences, Piscataway, NJ, USA) and according to the manufacturer's instructions. In brief, 2 μL (10-20 ng) of total Extracted DNA were dissolved in 5 μL of sample buffer, denatured for 3 min at 95°C and cooled down on ice for 1 min followed by addition of 5 μL reaction buffer and 0.2 μL enzyme mix. Amplification was performed

for overnight (16-20 h) at 30°C and the reaction was stopped by heating for 10 min at 65°C to inactivate the enzyme. The amplified DNA was used as a template for the PCR to amplify the full-length genome of tomato yellow leaf curl virus and to detect the occurrence of β satellite.

Full-length amplification of TYLCV-DNA-A: The DNA-A of TYLCV were amplified by PCR using the primer pairs (TYLCV1F 5-ACAGCCCATACAGCAGCCGTGC-3 and TYLCV1R 5-GCTGTCGAAGTTCAGCCTTCGGC-3). The PCR reaction contained 1 μL of RCA product, 0.25 mM $MgCl_2$, 2.5 μM of each primer (TYLCV1F/ TYLCV1R), 0.25 mM dNTPs, 1×enzyme buffer and 0.5 U of HotStar Taq Plus DNA polymerase (Qiagen) in a total volume of 25 μL. The viral DNAs were amplified using amplification program as following; one cycle of denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min and extension at 72°C for 2 min followed by final extension step at 72°C for 10 min. Amplified DNA fragments were electrophoresed in 0.7% agarose gel in TBE buffer and visualized using UV transilluminator after staining with ethidium bromide.

Amplification of DNA-β: To study the presence of DNA-β molecules in the TYLCV infected samples, two consensus outwardly extending primers were used (beta F1, 5-GTTGTCTTGT TTATTATGTTTGTTGGC-3 as forward primer; beta 02, 5-GGTACCTACCCTCCCAGGGGTACAC-3 (Briddon *et al.*, 2002) as reverse primer. The PCR reaction contained 1 μL of RCA product of template DNA, 15 mM $MgCl_2$ buffer, 10 pmol dNTPs, 40 pmol of each primer and 2.5 U Taq polymerase. The reactions were denatured at 94°C for 3 min, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, terminating with 10 min at 72°C.

Cloning and sequence analysis: The amplified fragments for DNA-A and DNA-β were ligated into pGEM-T easy cloning vector (Promega, WI, USA) and cloned according to the manufacturer's instructions. After transformation into the DH5α strain of *Escherichia coli*, white colonies were screened for the gene of interest by PCR and restriction digestion with EcoRI. General primers Sp6 and T7 were used to sequence the clones of DNA-A and DNA-β fragments in both directions (Macrogen Inc., Seoul, Korea). Alignment analysis were performed using the online BLAST service of the National Center for Biotechnology Information (URL:http//www.ncbi. nlm.nih.gov/BLAST/). The GC content for the two isolates in

this sudy was calculated using the Geecee software (http://weblab.cbi.pku.edu.cn/program.inputForm.do?Program=geecee) and SnapGene viewer software version 3.1.

Phylogenetic and homology analysis: Nucleotide sequences reported in this study were deposited in GenBank under the accession numbers KF435136 (isolate from *C. frutescens*) and KF444467 (isolate from *P. vulgaris).* Sequences were retrieved and BLAST analyzed (National Center for Biotechnology Information (URL:http//www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic trees were constructed with the DNAMAN software version 8 (Lynnon, Canada) usingthe neighbor-joining method (Saitou and Nei, 1987) and the Jukes-Cantor distance correction method (Jukes and Cantor, 1969).

RESULTS

Sampling and PCR amplification: Virus infected plants of common bean and pepper collected from the field showed yellowing and leaf curling symptoms (Fig. 1). The plants in early stage showed stunting and the production decreased. The relationship of a begomoviruses with the symptomatic *P. vulgaris* and *C. frutescens* was confirmed by PCR using AVcore/ACcore primers as degenerate primers for begomoviruses. The symptomatic *P. vulgaris* and *C. frutescens* samples gave bands of the expected size (~580 bp) (Fig. 2a). The RCA was performed to amplify all circular DNA molecules from the extracted DNA for the samples that gave amplicons with AVcore/ACcore primers. As in Fig. 2b the expected size (~2.8 kb) of the full length DNA-A genome was amplified from RCA *P. vulgaris* and *C. frutescens* DNA templates. In addition, a fragment (722 bp) of the DNA-β satellite genome was successfully amplified using RCA of *P. vulgaris* as template (Fig. 2c). This study never found any DNA-β satellite by RCA using *C. frutescens* as template.

Full genome sequences analysis: Full-length genome sequences of TYLCV-Pep from *C. frutescens* and TYLCV-GB from *P. vulgaris* were analyzed and compared with other known sequences in the DNA database that obtained from GenBank (Table 1). The DNA-B was not found by PCR in the two TYLCV isolates using different sets of specific primers. However, DNA-β satellite was found associated with TYLCV-GB when DNA-β specific primers were used. The size of the full length genomes of TYLCV-Pep and TYLCV-GB were 2764 and
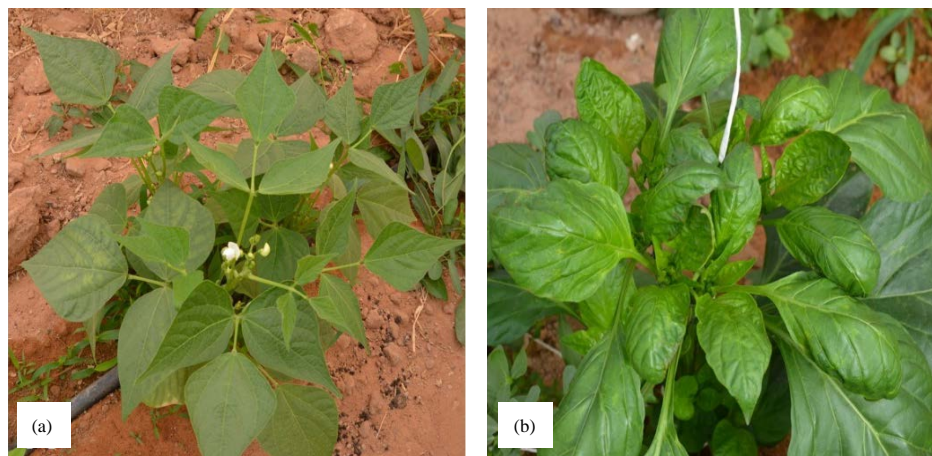
Fig. 1(a-b): (a) *Phaseolus vulgaris* and (b) *Capsicum frutescens* showing yellowing and leaf curling symptoms of *Begomovirus* infections
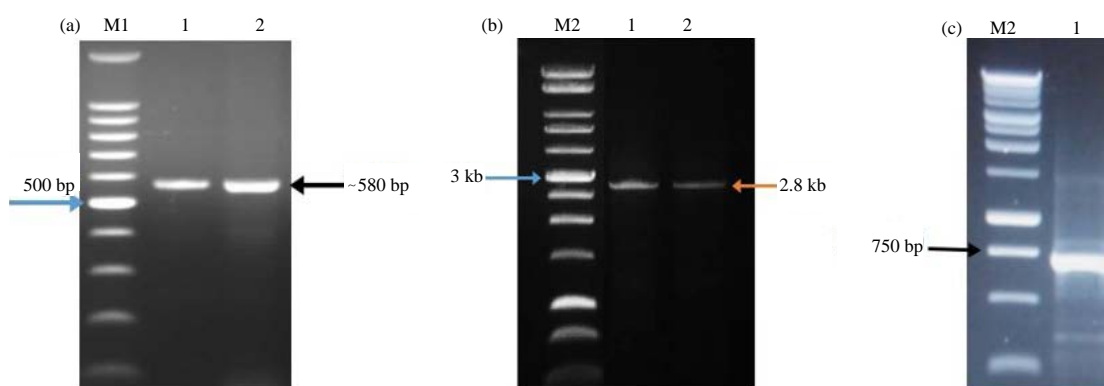

Fig. 2(a-c): Agarose gel electrophoresis of PCR amplified products of, (a) Partial coat protein gene, (b) Full-length genome of TYLCV and (c) Partial fragment of DNA-β from the RCA templates of TYLCV-GB and TYLCV-Pep isolates. Lanes M1 = 100 bp marker, M2 = 1 kb ladder, 1 = Sample from common bean (TYLCV-GB) and 2 = Sample from pepper (TYLCV-Pep)

Table 1: GenBank accession numbers for tomato yellow leaf curl virus sequences used for sequence analysis

Isolates	Accession No.	Countries	References
TYLCV-GB	KF444467	Saudi Arabia	This study
TYLCV-Pep	KF435136	Saudi Arabia	This study
TYLCV-Qas	KF561125	Saudi Arabia	Rezk and Alhudaib (2016)
TYLCV-Hail	KF040453	Saudi Arabia	Rezk and Alhudaib (2016)
TYLCV-Has	KF435137	Saudi Arabia	Rezk and Alhudaib (2016)
TYLCV-Jiz	KC845301	Saudi Arabia	Rezk and Alhudaib (2016)
TYLCV-KSA46	HG530539	Saudi Arabia	Idris *et al.* (2014)
TYLCV-Sudan	HE819244	Sudan	Khan *et al.* (2012)
TYLCV-Kahnooj1	GU076448	Iran	Lefeuvre *et al.* (2010)
TYLCV-Ir2	EU085423	Iran	Azizi *et al.* (2008)
TYLCV-Kahnooj2	GU076449	Iran	Lefeuvre *et al.* (2010)
TYLCV-Jazira	AY044137	Sudan	Idris and Brown (2005)
TYLCV-Mir-Sud	JN591385	Sudan	Khan *et al.* (2012)
TYLCV-Yemen	EF110891	Yemen	Isakeit *et al.* (2005)
TYLCV-Genaveh	GU076454	Iran	Lefeuvre *et al.* (2010)

2779 bp respectively (Fig. 3). The length of the IR was 290 bp for TYLCV-Pep and 305 bp for TYLCV-GB. The homology and phylogenetic analysis are shown in Fig. 4 and Table 2. The nucleotide identity between TYLCV-GB and TYLCV-Pep was 81.2%. The highest nt identity of TYLCV-GB was 96.9% with TYLCV-Hail, while the highest nt identity of TYLCV-Pep was 94% with TYLCV-Genaveh (isolate from Iran). As in Table 2, six Open Reading Frames (ORFs) were identified in the TYLCV-Pep genome (two in the viral strand and four in viral complementary strand) whereas seven ORFs were determined at the TYLCV-GB genome (two in the viral strand and five in the viral complementary strand). The number of amino acids translated from each ORF in the two isolates is shown in Table 2. The GC content was 42% in both
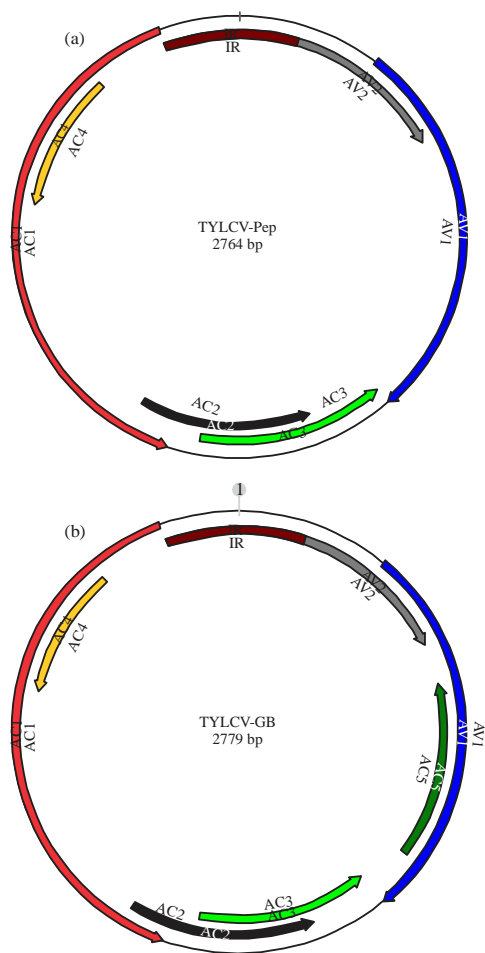
Fig. 3(a-b): Genome size and ORF organization of, (a) TYLCV-Pep and (b) TYLCV-GB using SnapGene viewer 3.1 software

TYLCV-GB and TYLCV-Pep; the GC content in all known begomoviruses ranges between 41-42% (Table 2). The IR identity value between TYLCV-GB and TYLCV-Pep was very
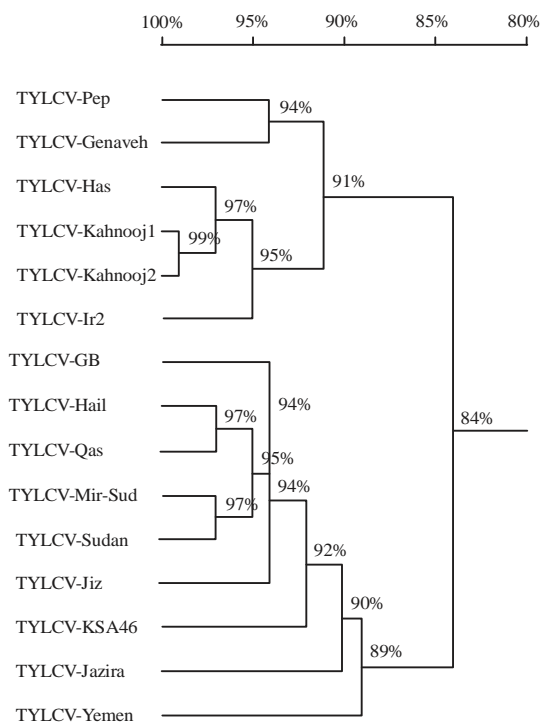

Fig. 4: Homology tree showing the identity percentage among the isolated Tomato Yellow Leaf Curl Virus (TYLCV-GB and TYLCV-Pep) and other strains and isolates selected from GenBank and were aligned with the optimal alignment method of DNAMAN

Table 2: Genomic characterization of TYLCV-GB and TYLCV-Pep and other isolate and strains that available in GenBank database

| | | Identity (%) | | | | No. of amino acids in each ORFs | | | | | | |
| | | | | | | Virion strand | | Complimentary strand | | | | |
Isolate	Genome length (bp)	TYLCV-GB	TYLCV-Pep	GC content	IR length	V1	V2	C1	C2	C3	C4	C5
TYLCV-GB	2779	-	-	0.42	305	256	116	358	134	133	95	128
TYLCV-Pep	2764	81.2	-	0.42	290	257	115	358	131	133	99	-
TYLCV-Qas	2775	94.0	82.8	0.42	208	243	115	389	134	113	97	128
TYLCV-Hail	2775	95.9	83.1	0.42	211	243	115	389	134	113	97	128
TYLCV-Has	2771	81.7	89.7	0.42	297	257	115	358	134	133	99	-
TYLCV-Jiz	2780	94.1	82.8	0.41	309	256	116	358	134	133	95	-
TYLCV-KSA46	2788	90.1	82.7	0.42	262	256	115	377	134	133	99	128
TYLCV-Sudan	2772	94.1	82.2	0.42	244	243	115	388	134	113	95	128
TYLCV-Kahnooj1	2770	82.6	89.5	0.42	214	257	115	386	134	133	96	-
TYLCV-Ir2	2776	82.0	90.0	0.42	292	257	115	357	134	133	99	-
TYLCV-Kahnooj2	2770	82.2	89.1	0.42	214	257	115	386	134	133	96	-
TYLCV-Jazira	2779	89.7	81.9	0.42	310	256	115	358	134	133	99	128
TYLCV-Mir-Sud	2773	93.5	82.4	0.42	246	243	115	377	134	113	95	128
TYLCV-Yemen	2781	88.5	82.7	0.42	312	256	115	358	134	133	99	128
TYLCV-Genaveh	2764	81.8	94.0	0.42	292	257	115	358	134	133	99	-

Fig. 5: Conserved region of stem-loop structure in the all Saudi TYLCV recorded in GenBank database

low (74.6%) whereas it was 98.6% between TYLCV-GB and TYLCV-Hail. Also this value was 94.5% between TYLCV-Pep and TYLCV-Genaveh. All the Saudi TYLCV isolates contain an identical nucleotide sequence of 42 nucleotides (AAAGCGGCCATCCGT-ATAATATTACCGGATGGCCGCGCCCC) inside the IR region (Fig. 5). This conserved region forms a characteristic stem-loop structure as in all begomoviruses.

Nucleotide sequence analysis of DNA-β: Two pairs of primers beta F1 and beta 02 were used to PCR-amplify 722 nucleotides of DNA β satellite from RCA products as described above. The PCR amplicons were cloned into pGEM-Teasy vector (Promiga) and multiple clones were obtained. One of them was selected and sequenced in both orientations. Alignments and phylogenetic analyses were performed using DNAMAN (version 8) and BLAST. The DNA-β satellite was found associated with TYLCV infected common bean plants but not with TYLCV infected pepper plants. The obtained sequence of DNA-β satellite shows genome arrangement typical of DNA-β satellites associated with TYLCV isolates from Yemen (Idris *et al.*, 2012), with identity not up to 90%. One ORF was identified in the complementary sense known as βC1, predicted to a protein of encode 117 amino acids.

DISCUSSION

There are a few reports on the incidence of TYLCV infecting pepper and beans. The reports showing susceptibility of *Capsicum* spp. to TYLCV are conflicting. The inoculation studies in the greenhouse with isolates from Jordan and Israel indicated that *C. annuum* was not a host for TYLCV (Mansour and Al-Musa, 1992; Polston *et al.*, 2006), in contrast, other a report from Spain indicated that *C. annuum* is indea a host for this virus (Reina *et al.*, 1999). In other reports, PCR followed by restriction enzyme analysis and nucleic acid hybridization were successfully used to detect TYLCV field-infected plants of *C. annuum* and *C. chinense* in the Dominican Republic and Cuba (Salati *et al.*, 2002; Quinones *et al.*, 2002) and Jamaica (Roye *et al.*, 1999), respectively. The PCR results showed that most of tested

C. frutescens plants were infected with TYLCV. This result supported the previous reports that referred to *Capsicum* spp. as a host for TYLCV. The first evidence for the susceptibility of *C. frutescens*, *C. baccatum* and *C. chinense* was reported by Polston *et al.* (2006). Yellowing, mottling and curling symptoms were associated with TYLCV infected pepper plants; however, previous studies stated that the infected plants were symptomless (Morilla *et al.*, 2005; Polston *et al.*, 2006). The observed symptoms may be produced by whitefly feeding damage especially when high numbers of whiteflies were observed on the plants in the field. Because whiteflies are able to transmit this virus from pepper to tomato and vice versa as described by Polston *et al.* (2006), it may be expected that the virus is transmitted from cultivated tomato of neighboring fields to pepper plants. *P. vulgaris* is known as a favorable host for many viruses, especially the geminiviruses. The first report of TYLCV infection in *P. vulgaris* was in Spain (Navas-Castillo *et al.*, 1999). In this study, the observed symptoms of infected plants showed yellowing and light curling unlike those severe curling symptoms described by Kamaal *et al.* (2015). Perhaps this is because the infection was at the early stage or because our isolate is different. Based on nucleotide sequence and genome length, TYLCV-Pep is shorter than TYLCV-GB and is the same as TYLCV-Genaveh (Lefeuvre *et al.*, 2010), whereas, TYLCV-GB is same as TYLCV-Jazira (Idris and Brown, 2005). The distinction of the genome length occurred in the IR region but not in the protein coding regions because the length of IR of TYLCV-Pep was 290 and 305 bp for the TYLCV-GB. Phylogenetic analysis (Kim *et al.*, 2011) is useful in studying epidemiology and evolutionary history of viruses. The phylogenetic tree in Fig. 4 showed that TYLCV-Pep and TYLCV-GB were separated into two clusters. The TYLCV-GB was clustered with TYLCV isolates from Saudi Arabia, Sudan and Yemen while TYLCV-Pep was clustered with isolates from Iran. This result suggests that TYLCV-GB may have originated from Sudan or Yemen but TYLCV-Pep may have originated from Iran. The identity between TYLCV-GB and TYLCV-Pep was 81.2% indicating that they are two deferent strains. Two ORFs were observed on the viral strand of the genome of both viruses. The V1 ORF

encodes the coat protein 256 aa in the case of TYLCV-GB and 257 aa in the case of TYLCV-Pep. The coat protein of the virus plays an important role in systemic infection, insect transmission and particle formation (Noris *et al.*, 1998; Kim *et al.*, 2011). The ORF2 V2 encodes a 116 aa in case of TYLCV-GB and 115 aa in case of TYLCV-Pep. V2 protein performs as a suppressor of RNA silencing (Zrachya *et al.*, 2007) and is important for plant infection (Wartig *et al.*, 1997). On the complementary strand of the genome, there are four ORFs in case of TYLCV-Pep and five ORFs in case of TYLCV-GB. These ORFs encoding to C1, C2, C3, C4 and C5 proteins. The C1 ORF encodes the REP (replicase-associated protein) which contains a NTP-binding motif (Laufs *et al.*, 1995) and plays a critical role in viral replication protein. It is 358 aa in both TYLCV-GB and TYLCV-Pep. The C2 protein is a transactivator protein (TrAP) for the expression of virion sense gene and a host range determination factor (Sunter *et al.*, 2001; Wartig *et al.*, 1997). The C2 protein has 134 aa in all TYLCV strains except TYLCV-Pep has 131 aa. The C3 protein has 133 aa; it is known as a replication enhancer (REn) (Settlage *et al.*, 2005), conserved in most TYLCV strains worldwide. The C4 protein is involved in inducing the disease symptoms (Rigden *et al.*, 1994), it has 95 aa in case of TYLCV-GB and 99 aa in case of TYLCV-Pep. The C5 protein (128 aa) is observed in TYLCV-GB and other some TYLCV strains and isolates of Saudi Arabia, Sudan and Yemen (Idris *et al.*, 2012). This potential protein identified on the complimentary strand of TYLCV-GB genome starts from nucleotide 976 to nucleotide 589 and ends with a TGA termination codon. The function of this potential gene is not recognized yet even though it is recorded in some other begomoviruses. The identity of IR sequence between TYLCV-GB and TYLCV-Pep is low (74.6%), TYLCV-GB has 15 nucleotide more than TYLCV-Pep and this may explain the variation of the genome length. The importance of the IR region due to the mutation frequency in this region is higher than the other coding regions. The IR is described as a hot spot for nucleotide disincorporation during the DNA viral replication (Font *et al.*, 2007) and the high level of *Begomovirus* population diversity might reproduce replication errors (Bull *et al.*, 2006; Zhang *et al.*, 2008). Many of the monopartite begomoviruses have been shown to be associated with two circular ssDNA satellite molecules, betasatellite (DNA-β) and alfasatellite (DNA-1) (Briddon and Stanley, 2006; Fiallo-Olive *et al.*, 2013). The DNA-β depends on their helper virus for replication and encapsidation. It encodes a gene (βC1) involved in systemic disease development and plays an important role in pathogenicity of their helper virus (Briddon *et al.*, 2003; Fauquet *et al.*, 2008).

The DNA-β satellite was found to be associated with TYLCV-GB and shared 90% similarity with the closest relatives that are DNA-β molecules from Yemen (JF919717) (Idris *et al.*, 2012). The amplified DNA-β has one gene (βC1) which encodes a 129 aa protein on the complementary strand. The presence of alfasatellite (DNA-1) did not examine in this research. The first report of DNA-β satellite with TYLCV species was associated with TYLCV from Oman (Khan *et al.*, 2008).

CONCLUSION

The distinction of genome length, low similarity of genome and IR sequences and the presence of C5 and DNA-β associated with TYLCV-GB but not in TYLCV-Pep-suggest that TYLCV-GB and TYLCV-Pep are two different strains in Saudi Arabia with different invasion routes.

ACKNOWLEDGMENT

REFERENCES

Adams, M.J., A.M.Q. King and E.B. Carstens, 2013. Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2013). Arch. Virol., 158: 2023-2030.

Alhudiab, K., W. Alaraby and A. Rezk, 2014. Molecular characterization of tomato yellow leaf curl disease associated viruses in Saudi Arabia. Int. J. Virol., 10: 192-203.

Antignus, Y. and S. Cohen, 1994. Complete nucleotide sequence of an infectious clone of a mild isolate of tomato yellow leaf curl virus (TYLCV). Phytopathology, 84: 707-712.

Azizi, A., J. Mozafari and M. Shams-Bakhsh, 2008. A new strain of tomato yellow leaf curl virus from Iran. National Center for Biotechnology Information, Bethesda, MD.

Briddon, R.W., S. Mansoor, I.D. Bedford, M.S. Pinner and K. Saunders *et al.*, 2001. Identification of DNA components required for induction of cotton leaf curl disease. Virology, 285: 234-243.

Briddon, R.W., S.E. Bull, S. Mansoor, I. Amin and P.G. Markham, 2002. Universal primers for the PCR-mediated amplification of DNA β: A molecule associated with some monopartite *Begomoviruses*. Mol. Biotechnol., 20: 315-318.

Briddon, R.W., S.E. Bull, I. Amin, A.M. Idris and S. Mansoor *et al.*, 2003. Diversity of DNA β, a satellite molecule associated with some monopartite *Begomoviruses*. Virology, 312: 106-121.

Briddon, R.W. and J. Stanley, 2006. Subviral agents associated with plant single-stranded DNA viruses. Virology, 344: 198-210.

Briddon, R.W., B.L. Patil, B. Bagewadi, M.S. Nawaz-ul-Rehman and C.M. Fauquet, 2010. Distinct evolutionary histories of the DNA-A and DNA-B components of bipartite *Begomoviruses*. BMC Evol. Biol., Vol. 10. 10.1186/1471-2148-10-97.

Brown, J.K., A.M. Idris, I. Torres-Jerez, G.K. Banks and S.D. Wyatt, 2001. The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of *Begomoviruses*. Arch. Virol., 146: 1581-1598.

Brown, J.K., C.M. Fauquet, R.W. Briddon, M. Zerbini, E. Moriones and J. Navas-Castillo, 2012. Family Geminiviridae. In: Virus Taxonomy: Classification and Nomenclature of Viruses, King, A.M.Q., M.J. Adams, E.B. Carstens and E.J. Lefkowitz (Eds.). Elsevier Academic Press, USA., pp: 351-373.

Bull, S.E., R.W. Briddon, W.S. Sserubombwe, K. Ngugi, P.G. Markham and J. Stanley, 2006. Genetic diversity and phylogeography of cassava mosaic viruses in Kenya. J. Gen. Virol., 87: 3053-3065.

Cui, X., X. Tao, Y. Xie, C.M. Fauquet and X. Zhou, 2004. A DNAβ associated with tomato Yellow leaf curl china virus is required for symptom induction. J. Virol., 78: 13966-13974.

Czosnek, H. and H. Laterrot, 1997. A worldwide survey of tomato yellow leaf curl viruses. Arch. Virol., 142: 1391-1406.

De Barro, P.J., S.S. Liu, L.M. Boykin and A.B. Dinsdale, 2011. *Bemisia tabaci*: A statement of species status. Annu. Rev. Entomol., 56: 1-19.

Fauquet, C.M., R.W. Briddon, J.K. Brown, E. Moriones, J. Stanley, M. Zerbini and X. Zhou, 2008. Geminivirus strain demarcation and nomenclature. Arch. Virol., 153: 783-821.

Fiallo-Olive, E., D.T. Chirinos, F. Geraud-Pouey, E. Moriones and J. Navas-Castillo, 2013. Complete genome sequences of two *Begomoviruses* infecting weeds in Venezuela. Arch. Virol., 158: 277-280.

Font, M.I., L. Rubio, P.V. Martinez-Culebras and C. Jorda, 2007. Genetic structure and evolution of natural populations of viruses causing the tomato yellow leaf curl disease in Spain. Virus Res., 128: 43-51.

Hanssen, I.M., M. Lapidot and B.P.H.J. Thomma, 2010. Emerging viral diseases of tomato crops. Mol. Plant-Microbe Interact., 23: 539-548.

Idris, A.M. and J.K. Brown, 2005. Evidence for interspecific-recombination for three monopartite begomoviral genomes associated with the tomato leaf curl disease from Central Sudan. Arch. Virol., 150: 1003-1012.

Idris, A.M., N.M. Abdullah and J.K. Brown, 2012. Leaf curl diseases of two solanaceous species in Southwest Arabia are caused by a monopartite *Begomovirus* evolutionarily most closely related to a species from the Nile Basin and unique suite of betasatellites. Virus Res., 169: 296-300.

Idris, A., M. Al-Saleh, M.J. Piatek, I. Al-Shahwan, S. Ali and J.K. Brown, 2014. Viral metagenomics: Analysis of *Begomoviruses* by illumina high-throughput sequencing. Viruses, 6: 1219-1236.

Isakeit, T., A. Idris and J. Brown, 2005. Tomato yellow leaf curl virus in tomato (*Lycopersicon esculentum*) in Texas, originating from transplant facilities. Plant Sciences, University of Arizona, Tucson, AZ., USA.

Jukes, T.H. and C.R. Cantor, 1969. Evolution of Protein Molecules. In: Mammalian Protein Metabolism, Munro, H.N. (Ed.). Academic Press, New York, pp: 21-132.

Kamaal, N., M. Akram and A.K. Agnihotri, 2015. Molecular evidence for the association of tomato leaf curl Gujarat virus with a leaf curl disease of *Phaseolus vulgaris* L. J. Phytopathol., 163: 58-62.

Khan, A.J., A.M. Idris, N.A. Al-Saady, M.S. Al-Mahruki, A.M. Al-Subhi and J.K. Brown, 2008. A divergent isolate of tomato yellow leaf curl virus from Oman with an associated DNAβ satellite: An evolutionary link between Asian and the Middle Eastern virus-satellite complexes. Virus Genes, 36: 169-176.

Khan, A.J., A.R.M.O. Al-Matroushi and S. Akhtar, 2012. Diversity of *Begomoviruses* on field crops in Oman. Sultan Qaboos University, Oman.

Kil, E., J. Park, H. Lee, J. Kim and H.S. Choi *et al.*, 2014. *Lamium amplexicaule* (Lamiaceae): A weed reservoir for Tomato Yellow Leaf Curl Virus (TYLCV) in Korea. Arch. Virol., 159: 1305-1311.

Kim, S.H., S. Oh, T.K. Oh, J.S. Park and S.C. Kim *et al.*, 2011. Genetic diversity of tomato-infecting Tomato Yellow Leaf Curl Virus (TYLCV) isolates in Korea. Virus Genes, 42: 117-127.

Lapidot, M. and M. Friedmann, 2002. Breeding for resistance to whitefly-transmitted geminiviruses. Ann. Applied Biol., 140: 109-127.

Laufs, J., W. Traut, F. Heyraud, V. Matzeit, S.G. Rogers, J. Schell and B. Gronenborn, 1995. *In vitro* cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. Proc. Natl. Acad. Sci. USA., 92: 3879-3883.

Lefeuvre, P., D. Martin, G. Harkins, P. Lemey and A.J.A. Gray *et al.*, 2010. The spread of tomato yellow leaf curl virus from the Middle East to the world. PLoS Pathogens, Vol. 6. 10.1371/journal.ppat.1001164

Mansour, A. and A. Al-Musa, 1992. Tomato yellow leaf curl virus: host range and virus-vector relationships. Plant Pathol., 41: 112-125.

Martinez-Ayala, A., S. Sanchez-Campos, F. Caceres, L. Aragon-Caballero, J. Navas-Castillo and E. Moriones, 2014. Characterisation and genetic diversity of pepper leafroll virus, a new bipartite begomovirus infecting pepper, bean and tomato in Peru. Ann. Applied Biol., 164: 62-72.

Melgarejo, T.A., T. Kon, M.R. Rojas, L. Paz-Carrasco, F.M. Zerbini and R.L. Gilbertson, 2013. Characterization of a new world monopartite *Begomovirus* causing leaf curl disease of tomato in Ecuador and Peru reveals a new direction in *Geminivirus* evolution. J. Virol., 87: 5397-5413.

Morilla, G., D. Janssen, S. Garcia-Andres, E. Moriones, I.M. Cuadrado and E.R. Bejarano, 2005. Pepper (*Capsicum annuum*) is a dead-end host for tomato yellow leaf curl virus. Phytopathology, 95: 1089-1097.

Navas-Castillo, J., S. Sanchez-Campos, J.A. Diaz, E. Saez-Alonso and E. Moriones, 1999. Tomato yellow leaf curl virus-is causes a novel disease of common bean and severe epidemics in tomato in Spain. Plant Dis., 83: 29-32.

Noris, E., A.M. Vaira, P. Caciagli, V. Masenga, B. Gronenborn and G.P. Accotto, 1998. Amino acids in the capsid protein of tomato yellow leaf curl virus that are crucial for systemic infection, particle formation and insect transmission. J. Virol., 72: 10050-10057.

Papayiannis, L.C., A. Paraskevopoulos and N.I. Katis, 2007. First report of tomato yellow leaf curl virus infecting common bean (*Phaseolus vulgaris*) in Greece. Plant Dis., 91: 465-465.

Polston, J.E., L. Cohen, T.A. Sherwood, R. Ben-Joseph and M. Lapidot, 2006. *Capsicum* species: Symptomless hosts and reservoirs of tomato yellow leaf curl virus. Phytopathology, 96: 447-452.

Quinones, M., D. Fonseca, Y. Martinez and G. Accotto, 2002. First report of tomato yellow leaf curl virus infecting pepper plants in Cuba. Plant Dis., 86: 73-73.

Reina, J., G. Morilla, E.R. Bejarano, M.D. Rodriguez and D. Janssen, 1999. First report of *Capsicum annuum* plants infected by tomato yellow leaf curl virus. Plant Dis., 83: 1176-1176.

Rigden, J.E., L.R. Krake, M.A. Rezaian and I.B. Dry, 1994. ORF C4 of tomato leaf curl geminivirus is a determinant of symptom severity. Virology, 204: 847-850.

Roye, M.E., M.E. Wernecke, W.A. McLaughlin, M.K. Nakhla and D.P. Maxwell, 1999. Tomato dwarf leaf curl virus, a new bipartite *Geminivirus* associated with tomatoes and peppers in Jamaica and mixed infection with tomato yellow leaf curl virus. Plant Pathol., 48: 370-378.

Saitou, N. and M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425.

Salati, R., M.K. Nahkla, M.R. Rojas, P. Guzman, J. Jaquez, D. Maxwell and R.L. Gilbertson, 2002. Tomato yellow leaf curl virus in the dominican republic: Characterization of an infectious clone, virus monitoring in whiteflies and identification of reservoir hosts. Phytopathology, 92: 487-496.

Sanchez-Campos, S., A. Martinez-Ayalaa, B. Marquez-Martin, L. Aragon-Caballero, J. Navas-Castillo and E. Moriones, 2013. Fulfilling Koch's postulates confirms the monopartite nature of tomato leaf deformation virus: A *Begomovirus* native to the new world. Virus Res., 173: 286-293.

Segundo, E., M.P. Carmona, E. Saez, L. Velasco and G. Martin *et al.*, 2008. Occurrence and incidence of viruses infecting green beans in South-Eastern Spain. Eur. J. Plant Pathol., 122: 579-591.

Settlage, S.B., R.G. See and L. Hanley-Bowdoin, 2005. *Geminivirus* C3 protein: Replication enhancement and protein interactions. J. Virol., 79: 9885-9895.

Sunter, G., J.L. Sunter and D.M. Bisaro, 2001. Plants expressing tomato golden mosaic virus *AL2* or beet curly top virus *L2* transgenes show enhanced susceptibility to infection by DNA and RNA viruses. Virology, 285: 59-70.

Wartig, L., A. Kheyr-Pour, E. Noris, F. de Kouchkovsky, F. Jouanneau, B. Gronenborn and I. Jupin, 1997. Genetic analysis of the monopartite tomato yellow leaf curl *Geminivirus*: Roles of V1, V2 and C2 ORFs in viral pathogenesis. Virology, 228: 132-140.

Zhang, Y., W. Zhu, H. Cui, Y. Qiu and K. Sha *et al.*, 2008. Molecular identification and the complete nucleotide sequence of TYLCV isolate from Shanghai of China. Virus Genes, 36: 547-551.

Zrachya, A., P.P. Kumar, U. Ramakrishnan, Y. Levy and A. Loyter *et al.*, 2007. Production of siRNA targeted against TYLCV coat protein transcripts leads to silencing of its expression and resistance to the virus. Transgenic Res., 16: 385-398.